(19) JAPANESE PATENT OFFICE

PATENT ABSTRACTS OF JAPAN

(11) Publication number: **04327274 A**
(43) Date of publication of application: **16.11.1992**

(51) Int. Cl **D06M 15/00**
D06M 10/10, D06M 15/643
// D06M101:10

(21) Application number: **03122693**
(22) Date of filing: **24.04.1991**

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(54) **SHRINK PROOFING METHOD FOR ANIMAL HAIR FIBER PRODUCT**

(57) Abstract:

PURPOSE: To obtain a shrink-proof fiber having shrink proofing property and excellent resistance to washing and capable of retaining soft handle having drape property by subjecting woven and knitted fabric consisting of animal hair fiber product to specific shrink proofing.

CONSTITUTION: A woven and knitted fabric consisting of an animal hair fiber is subjected to low-temperature plasma treatment and then subjected to shrink proofing using e.g. blocked urethane resin and glyoxazal resin and subjected to weight reducing treatment by a proteolytic enzyme such as protease and as necessary subjected to softening treatment by an amino-modified dimethylsiloxane to provide an animal hair fiber product having shrink-proof performance and excellent washing resistance and applied in shrink proofing having soft handle.

(12) UK Patent Application (19) GB (11) 2 250 289 (13) A

(43) Date of A publication 03.06.1992

(21) Application No 9123714.9

(22) Date of filing 07.11.1991

(30) Priority data
(31) 4035839 (32) 10.11.1990 (33) DE

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(51) INT CL[6]
C12N 9/48, C14C 1/04 1/06 1/08

(52) UK CL (Edition K)
C3H HHX1 HK4 H517 H525 H526
C6C C2J3
U1S S1430

(56) Documents cited
GB 1403257 A DE 0128419 A

(58) Field of search
UK CL (Edition K) C3H HHX1 HK4 HP, C6C C2J3
INT CL[5] C12N, C14C
Online databases: WPI; BIOTECH (DIALOG)

(54) Preparations containing protease-tannin complex

(57) A substantially surfactant-free, powdered or granulated enzyme preparation comprises a protease in the form of a precipitated tannin complex, and at least 50 and up to 99.9% by weight of one or more salts conventionally used as a filler, extender or blending agent e.g. ammonium sulphate, sodium sulphate. The preparations may be used for the soaking, bating and dehairing of hides in leather manufacture.

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Enzyme Preparation

The invention relates to an enzyme preparation, in particular a surfactant-free solid enzyme preparation comprising protease an active enzyme obtained by tannin precipitation for the soaking, bating and dehairing of hides in leather manufacture.

For a long time, the technique of tannin precipitation has been an alternative method occasionally used in isolating enzymes from solutions, e.g. from vegetable juices or aqueous culture media. There is relatively extensive prior art on this subject (see for example GB-A-1156900, DE-A-1642619). The objective is generally to purify the enzymes. Additional measures for achieving good precipitation include the addition of gelatine and working in an acid pH range (pH 3-5). However, since tannin is harmful to subsequent enzyme preparations - finally forming an insoluble complex with the enzymes which it is supposed to precipitate - it has to be removed again, usually by treating the precipitate with organic solvents, e.g. acetone or ethanol, or by adding surfactant or raising the pH in the tannin precipitation; these are all procedures which can generally only be carried out on a laboratory scale without major complications.

The technical use of tannin complexes themselves has been described in just a few cases. Thus, a tannin complex is processed together with skimmed milk by drying to form a stable α-amylase preparation (CS 141 233). For medical use, an insoluble pancreas preparation is provided, produced by precipitating pancreas using tannic acid. The preparation is insoluble in the acidic gastric juices but develops its effect in the alkaline intestinal sections (DE 128 419). Moreover, DE-A-2143945 describes "water-insoluble dried

enzyme adducts tolerated by the skin", in the form of tannin complexes, inter alia. For practical use it is envisaged that the adduct of a bacterial proteinase obtained from tannin precipitation be incorporated directly in the detergent powder. Since the tannin complex is a fully active detergent, in this particular application it must be "used up" in the washing liquor.

As can be seen from the above description of the prior art, the process of tannin precipitation of enzymes from an aqueous medium is indeed a method of precipitation which can be used, but any industrial use of these precipitated enzyme-tannin complexes appears to be ruled out - apart from the exceptions mentioned - by their relative stability under potential conditions of use.

In order to prepare solid enzyme preparations in leather manufacture, particularly proteinase-containing preparations for use as bating and soaking preparations, it has hitherto been customary to use precipitation by means of ammonium sulphate or sodium sulphate from pressed pancreatic juices or bacterial culture juices (cf. Ullmanns Encyclopädie der technischen Chemie, 4th Edition, Volume 10, pages 475-561, page 495 ff, Verlag Chemie, 1975, DE-A 22 34 412).

However, particularly from an ecological point of view, this procedure is by no means ideal and generally causes substantial pollution of the waste water. This is clear from the usual rule of thumb which says that 50 kg of ammonium sulphate are required per 100 litres of enzyme-containing juice for precipitation. Use of such a large quantity of salt runs directly contrary to the present trend in waste water management, which is to restrict the sulphate content as far as possible. Rather, efforts should be made to find an efficient enzyme precipitation method in which the waste water pollution is substantially less and which, in the final analysis, should also be cheaper, particularly with

respect to the leather industry which operates under considerable pressure on costs.

It has now been found that the proteinase preparations according to the invention address these problems. The invention provides a substantially surfactant-free, powdered or granulated enzyme preparation comprising a protease present as a tannin complex, preferably obtained by precipitation of the said protease present in an aqueous medium by the addition of tannin, and at least 50 wt.-%, preferably more than 90 wt.-% and up to 99.9 wt.-% of one or more salts used as a filler, extender or blending agent.

The term "tannins" in the context of the present invention refers to the polyphenols categorised under this name, generally those which occur naturally, especially the tannic acids. (Further information can be found in Ullmann, Encyclopädie der Techn. Chemie, 3rd Edition, Volume 11, page 593-594; Kirk-Othmer, Encyclopedia of Chemical Technology, 2nd Ed. Vol. 12, pp. 319 - 325, J. Wiley 1967, Zechmeister Hersg. Fortschritte der Chemie organischer Naturstoffe, Vol. 41, 1 - 46, Springer Verlag).

The term "proteases" comprises the enzymes listed under E.C.3.4. (see Kirk-Othmer, Encyclopedia of Chemical Technology, 3rd Ed., Vol. 9, 173 - 223; J. Wiley 1980; E. Pfleiderer, R. Reiner in H.J. Rehm & G. Reed, Biotechnology, Vol. 6b, 729 - 742, VCH 1988; K. Aunstrup in B. Spencer Ed. Industrial Aspects of Biochemistry, Vol. 30 (I), pp. 23 - 46, North Holland, 1974). Various distinguishing criteria are used, including the criterion for distinguishing by origin:

a) animal origin, such as

   α) Rennin (E.C.3.4.23.4)
   β) pancreas proteases
      pancreatin, particularly trypsin, chymotrypsin

(pH-activity range about 7 - 10)
pepsin (E.C.3.4.23.1) (pH-activity range about 1.5 - 4.0) cathepsin (E.C.3.4.23.5) pH-activity range about 4.0 - 5.0)

b) plant origin

α) papain (E.C.3.4.22.1) pH-activity range about 5.0 - 8.0
β) ficin (E.C.3.4.22.3) pH-activity range about 4.0 - 9.0
γ) bromelain (E.C.3.4.22.4 and 3.4.22.5) pH-activity range about 5.0 - 7.0

c) microbial origin (see L. Keay in "Process Biochemistry" 1971; pages 17 - 21).

α) from types of Bacillus such as B.subtilis, B.licheniformis, B.alkalophilus, B.cereus, B.natto, B.vulgatus, B.mycoides.
β) from types of Streptococcus
γ) from types of Streptomyces such as Streptomyces fradiae, S.griseus, S.rectus
δ) from types of Aspergillus such as Aspergillus flavus-oryzae, A.niger, A.saitoi, A.usamii
ε) from types of Mucor and Rhizopus such as Mucor pusillus, M.miehei
ζ) types of Endothia such as Endothia parasitica
η) types of Trametes such as Trametes sanguinea

Apart from distinguishing by origin, a distinction is also made according to the site of attack (exo-versus endo-enzymes) and according to the "active site" of the proteases (serine proteases, inhibited by DFP, sulfhydryl enzymes). The pH dependency of the enzyme activity is also of major practical importance. A distinction is therefore made primarily from a practical

point of view between

i) alkaline proteases with an optimum activity in the range from pH 7.5 to 13, particularly alkaline bacterial proteases (E.C.3.4.21.) (which are usually of the serine type) and alkaline fungal proteases

ii) neutral proteases with an optimum activity in the range from pH 6.0 - 9.0, particularly neutral bacterial protease (E.C.3.4.24) (which belongs to the metalloenzymes) and fungal proteases, e.g. Bacillus proteases, Pseudomonas proteases, Streptomyces proteases, Aspergillus proteases.

iii) acid proteases with an optimum activity in the range from pH 2.0 - 5.0 (E.C.3.4.23) particularly acid fungal proteases, e.g. from Rhizopus spp., Aspergillus spp., Penicillium spp., Mucor spp., and Impex lacteus and Endothia parasitica.

As examples of alkaline proteases, particularly preferred are the subtilisines, alkaline bacterial proteinases of the serine type which are stable at pH 9 to 10 and to some extent unaffected by perborate.

The use of proteolytic enzymes has been an established part of leather manufacture for around 80 years, particularly in the beamhouse, since the introduction of enzymatic bating (tryptic digestive enzymes of the pancreas) in the OROPON® bate by Dr. Otto Röhm (German Patent No. 200519):
In addition to being used in bate (DE-B-927464; DE-B-976107; DE-B-941811; DE-B-974813, DE-B-975095; DE-B-976928; DE-B-1120066; DE-B-1134474; DE-B-1219620; DE-B-1282837; US-A-3939040; US-A-4273876) enzyme preparations are also used in soaking (DE-B-288095; DE-B-976662; DE-B-1022748; DE-B-1034317; DE-B-1282828;

DE-B-2059453; US-A-4278432; US-A-4344762) and also in hair loosening and opening up the hides (US-A-4294087).

The proteolytic activity of enzymes is conventionally measured by the Anson-Haemoglobin Method (M.L. Anson J. Gen. Physiol. 22, 79 (1939)) or by the Löhlein-Volhard method (the Löhlein-Volhard method for determining proteolytic activity, Gerbereichem. Taschenbuch. Dresden-Leipzig, 1955) in "LVU" (Löhlein-Volhard Units). A Löhlein-Volhard Unit is the quantity of enzyme which digests 1.725 mg of casein under the specific conditions of the method. (See R. J. Beynon, J.S. Bond, Proteolytic Enzymes IRL press).

Units derived from the Anson method are also used hereinafter for determining the activity of the enzymes which are effective in the acid range. These units are referred to as "proteinase units" (haemoglobin U_{Hb}). A U_{Hb} corresponds to the quantity of enzyme which catalyses the release of fragments of haemoglobin, soluble in trichloroacetic acid, equivalent to 1 μmol of tyrosine per minute at 37°C (measured at 280 nm). (1 mU_{Hb} = 10^{-3} U_{Hb}).

As already mentioned, the proteinase preparations according to the present invention are advantageously prepared directly from aqueous culture media or enzyme-containing juices. The recovery of proteinase preparations from the pancreas is of particular interest.

A. Recovery from the pancreatic complex

The procedure used according to the invention may partly conform to isolation methods used in the prior art (cf. Ullmann, 4th Edition, Volume 10, loc.cit. pages 536 - 537).

The enzymes are advantageously isolated from the pancreas immediately after slaughtering, predominantly from pigs or cattle. About 100 pancreases can be

processed in a batch, for example, by removing fat and connective tissue from the glands as far as possible immediately after slaughtering and then homogenising the gland tissue, e.g. using a mincer.

Immediately afterwards, extraction is appropriately carried out with about twice the volume (about 60 litres) of 0.25 N sulphuric acid at 5°C for 18 to 24 hours. After the addition of filter flakes, the mixture is advantageously pressed through a packing press. The pressing plates can be discarded. The substantially fat-free pressed juice thus obtained forms the starting material for recovering the proteinase preparations.

B. Recovery from other proteinase-containing aqueous crude extracts

Instead of the pressed pancreas juices, other proteinase-containing culture juices, e.g. from fungal or bacterial cultures, may be used (see the remarks above on the enzyme procedures and Ullmann, 4th Edition, Volume 10, pages 518 - 522, H.J. Rehm, G. Reed Ed. Biotechnology Volume 7a, pages 156 - 168, VCH 1987). As a rough guide, the culture juices may contain between 0.01 and 3% by weight of protein. The surfactant-free, solid enzyme products in powdered or granulated form containing the proteases as active enzymes may be obtained as follows:

First of all, correct metering of the precipitating agent has proved important. If insufficient tannin is used, the precipitation obtained is incomplete, whereas if too much is used the tannin complex formed is not readily used up. A useful rule is to use precisely enough precipitating agent to produce 0.5 to 3% enzyme reactivity in the supernatant.

As, for example, in precipitation from pressed pancreas juices (containing about 2 wt.-% of active protein) 4 wt-% of mimosa tannin are advantageously

used. Generally, the protein content in the tannin complexes is conveniently from 10 to 80 wt.-%.

Specifically, it is advantageous to establish a low pH for the aqueous juices (although obviously the stability of the proteases in question must be ensured). A pH of about 3 to 6 is a rough guide, with culture juices and pancreas generally having a pH of about 6. Precipitation above pH 7 is less satisfactory. In any case, it is advantageous to use lower temperatures ($\leq 20°C$). The addition of water-insoluble surfactants with an HLB value of < 6 has also proved favourable. Such surfactants are selected, for example, from the category of long-chained esters such as, for example, sorbitan fatty acid ester. Mention may be made, for example, of sorbitan-monooctadecanoate and the like. Surfactants of this kind are commercially available (see for example products made by Atlas Chemie, Essen, Germany of the SPAN® type).

By definition, a content of the enzyme preparations of at least 50 wt.-% of one or more salts normally used as extenders is envisaged. These salts are preferably ammonium sulphate or sodium sulphate.

Advantageous effects are already recorded from the point of view of manufacture and handling but not least in the use of the enzyme preparations according to the invention.

The preparation of the tannin complexes comprises a very good use of resources since it is a highly effective and, at the same time, selective method of precipitation. Particular mention should be made of the substantially reduced environmental pollution caused by the "mother liquors" compared, for example, with the salt precipitation which is usually carried out. The enzyme preparations can also be regarded as "progressive" from the point of view of hygiene as they are not dusty and hardly ever have an allergenic effect on the skin.

From the point of view of applications, it is advantageous that the enzyme preparations according to the invention can be used within the scope of conventional technical practice, i.e. there is no need for any fundamental change in the procedures used in the beamhouse. The use of the enzyme preparations according to the invention has proved particularly useful within the traditional, enzymatically aided soaking and bating operations. A high pH has proved particularly favourable (pH > 8) since this favours the "using up" of the tannin complexes.

The preparation according to the invention may be used in the soaking of hide material The soaking of hide material, during which the hardening of the skin which occurs with salting is reversed, is usually carried out at pH > 7.0 to 10.0. The simultaneous use of enzymes, particularly proteolytic enzymes, accelerates the soaking action by "digesting" the water-soluble and other proteins which do not belong to the collagen fibre structure of the hide. Generally, the soaking operation uses enzymes with an activity range (or optimum pH for proteolytic activity) at pH 7.0 to 10.0. Once the non-collagen proteins are removed, faster and more intensive wetting of the hide is guaranteed. Preferably, the soaking water is made alkaline (see above), but the pH should always remain below 12. It is also beneficial to use soaking adjuvants (such as non-ionogenic and anionic surfactants in conjunction with substituted phenols or dithiocarbamates in the usual concentration ranges (0.1 to 10 g/l).

Enzymatic additives used in the enzyme formulations according to the invention may be, for example, the above-mentioned proteases, particularly those listed under c), especially microbial proteases active in the range from 7 to 11.0, particularly Bacillus proteases, Streptomyces proteases and fungal proteinases, e.g.

those obtained from types of Aspergillus such as A.saitoi and A.usamii, and those from A.oryzae with a pH activity range of 7.0 to 9.5, in addition to those obtained from A.niger and A.flavus with a pH activity range of from 9.5 to 11.0. Thus, the initial pH in the enzymatic soak should preferably be 7.0 to 11.0, particularly 8.0 to 11.0.

Generally, the concentration of proteolytic activities of the proteinases used is in the range from 0.01 to 0.03 Anson units or 1000 to 3000 Löhlein-Volhard units per litre of soaking liquor. The quantities of enzyme preparation EP used will therefore correspond to these concentrations, according to their enzyme content. Finally, the soaking liquors may also contain amylases. The amylases occur for example as accompanying enzymes of fungal proteinases. They promote the cleaving of glycoside bonds in the proteoglycanes and glycoproteins of the skin. The soak is generally followed, at the end of the beamhouse stage, by the liming process, followed by deliming and bating, usually enzymatic bating.

The enzyme preparations may also be used to advantage in these following operations.

The preparations according to the present invention may also be used in bating. Deliming is traditionally used to reduce the alkalinity of the hides from pH levels of 13 to 14 to pH levels in the range from 7 to 8. Deliming should preferably be carried out, not with strongly dissociated but with weak organic acids, e.g. of the dicarboxylic acid type or slightly acidic salts. Bating is intended to remove residues of epidermis, hair and pigment and achieve additional opening up of the hide. It also removes any non-collagen protein constituents (see Ullmann, 4th Edition, Vol. 16 loc.cit. pages 119 - 120). Bating is conventionally carried out at pH 7.5 to 8.5. DE-A-3108428 discloses the use of cyclic carbonates in the deliming process. At the same time, lipases, e.g. pancreas lipases with an activity

range of pH 7.0 to 9.0 may also be used in the bate. Amylases according to B. (above), for example pancreas amylases, with an activity range of pH 5.5 to 8.5, which favour the cleaving of glycoside bonds during bating, also have a favourable effect on the bating process (particularly as accompanying enzymes of trypsin and chymotrypsin). Thus, the initial pH in the enzymatic bate should preferably be >5 to 11, particularly >6 to 9.

The Examples which follow are intended to illustrate the invention.

EXAMPLES

Example 1

Production of a pancreas preparation

100 kg of filtrate from degreased pancreas glands containing 1.4% protein are mixed with a solution of 5 kg of mimosa tannin in 20 kg of water, with stirring, at ambient temperature. The immediate precipitation of protein is stirred for a further 2 hours at ambient temperature (20°C) and then filtered. The filter cake is pressed in a packing press and then pre-comminuted to some extent. It is then gently dried at temperatures of 60 to 80°C and mixed with ammonium sulphate in the ratio 1:10.

The mixture produced can be used directly in the soaking and bating of leather, for dehairing and for opening up wet-blues.

Example 2

Production of a preparation from an A.oryzae fermentation

A proteinase concentrate containing about 2% protein is obtained from a fungal culture by ultrafiltration and cooled to +15°C. In order to improve the precipitation qualities, 100 kg of the concentrate are mixed with 100 g of SPAN®40 (made by Atlas Chemie, Essen, Germany) and then with 4 kg of mimosa tannin in 16 kg of water. Immediately, the protein begins to precipitate. After standing for 1 hour, the mixture, together with 2 kg of filter aid based on Perlit (DICALITE®4408, made by Dicalite of Neuss), is filtered (60 - 80 litres per hour and per m^2 of filter surface). The filter cake, having been

squeezed out, is carefully dried (60 to 80°C) and mixed in the ratio 1 : 30 with a mixture of sodium sulphate and ammonium sulphate.

The mixture obtained may be used directly in the soaking and bating of leather, for dehairing and for opening up wet-blues.

CLAIMS

1. A substantially surfactant-free, powdered or granulated enzyme preparation comprising protease present as a tannin complex and from 50 to 99.9% by weight of one or more salts used as a filler, extender or blending agent.

2. An enzyme preparation as claimed in claim 1 wherein said filler, extender or blending agent is ammonium sulphate or sodium sulphate.

3. An enzyme preparation as claimed in claim 1 substantially as herein described with reference to the Examples.

4. The use of an enzyme preparation as claimed in any one of the preceding claims in an enzymatic soak.

5. The use of an enzyme preparation as claimed in any one of claims 1 to 3 in an enzymatic bate.

6. The use of an enzyme preparation as claimed in claim 4 wherein the initial pH is from 7 to 11.

7. The use of an enzyme preparation as claimed in claim 6 wherein the initial pH is from 8 to 11.

8. The use of an enzyme preparation as claimed in claim 5 wherein the initial pH is from > 5 to 11.

9. The use of an enzyme preparation as claimed in claim 8 wherein the initial pH is from > 6 to 9.

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Patents Act 1977
Examiner's report to the Comptroller under Section 17 (The Search Report)

Application number

9123714.9

Relevant Technical fields

(i) UK Cl (Edition K) C3H (HHX1;HK4;HP), C6C (C2J3)

(ii) Int Cl (Edition 5) C12N; C14C

Search Examiner

MS N R CURTIS

Databases (see over)

(i) UK Patent Office

(ii) ONLINE DATABASES: WPI; BIOTECH (DIALOG)

Date of Search

3 FEBRUARY 1992

Documents considered relevant following a search in respect of claims 1-9

Category (see over)	Identity of document and relevant passages	Relevant to claim(s)
X	GB 1403257 KALI-CHEMIE AKTIENGESELLSCHAFT See aprticularly Example 1 and 2 column 2, lines 76-83	1, 2
X	DE 128419 DR FRANZ THOMAS & DR W WEBER IN STOLBERG	1, 2

Category	Identity of document and relevant passages	Relevant to claim(s)

Categories of documents

X: Document indicating lack of novelty or of inventive step.

Y: Document indicating lack of inventive step if combined with one or more other documents of the same category.

A: Document indicating technological background and/or state of the art.

P: Document published on or after the declared priority date but before the filing date of the present application.

E: Patent document published on or after, but with priority date earlier than, the filing date of the present application.

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